A Systematic Evaluation of the Bleomycin A_2 L-Threonine Side Chain: Its Role in Preorganization of a Compact Conformation Implicated in Sequence-Selective DNA Cleavage

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Abstract: The preparation and examination of **3**−**7** are detailed and constitute analogues of deglycobleomycin A_2 (**2**) containing systematic modifications in the L-threonine side chain. The studies revealed a substantial impact of the substituent on the DNA cleavage efficiency and ratio of double strand/single strand (ds/ss) cleavage without affecting the characteristic 5′-GPy selectivity. The results of the comparisons proved consistent with conformational effects of the substituent within the linker domain which restrict the number of accessible conformations ($\Phi \cong -120°$, $\Psi = 60-180°$) and favor adoption of a compact conformation ($\Phi \cong -120°$, $\Psi \cong 180°$) implicated in sequence-selective DNA cleavage. The studies also identify one potential site that may adopt two nearly equivalent turn conformations. This site may constitute one swivel point in the structure that permits access to a class of related bound structures ($\Psi = 60-180°$) adaptable to variable conformational characteristics required of multiple cleavage sites, including access to both strands of duplex DNA from a single intercalation site important for both primary and secondary DNA cleavage.

Bleomycin A_2 (**1**, Figure 1),[1−13] the major constituent of the clinical antitumor drug Blenoxane, is thought to derive its therapeutic effects from the ability to mediate the oxidative cleavage of double-stranded DNA or RNA by a metal ion- and oxygen-dependent process.[14−20] Studies employing derivatives of the natural product,[3,21,22] its degradation products or semi-

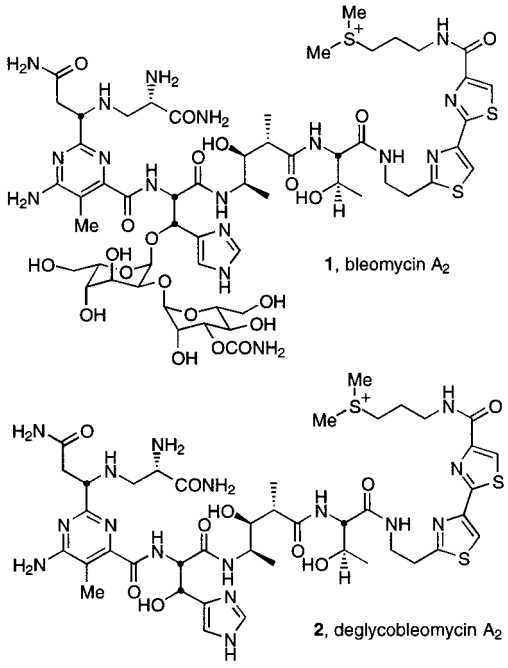

Figure 1.

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synthetic analogues,[23−32] as well as closely related or substantially simplified analogues,[33−36] have contributed to an emerging model of the structural features responsible for the sequence-selective cleavage of duplex DNA. In our own efforts,[37−49] this has entailed single point changes in the structure of bleomycin A_2 or deglycobleomycin A_2 (**2**), conducted with the intention of defining the role of each subunit, functional group, or substituent. These studies, carried out in conjunction with structural studies, have begun to unravel many of the subtle structural features contributing to the properties of the natural product.

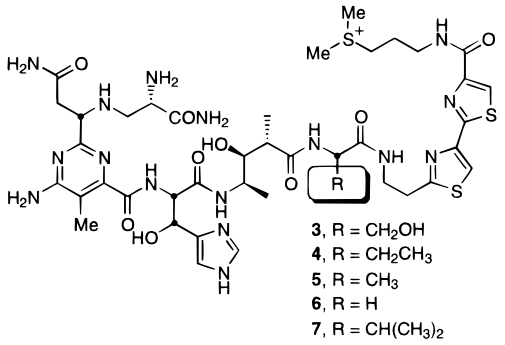

Figure 2.

Herein we report the synthesis and evaluation of **3**−**7** in which the L-threonine side chain of **2** has been systematically modified in efforts to dissect the subtle contributions of this subunit (Figure 2). In an initial comparison involving substantial modifications in the linker region of deglycobleomycin A_2 (**2**), the replacement of the L-threonine subunit with glycine (**6**) was found to have no impact on the characteristic 5′-GT, 5′-GC cleavage selectivity but did have a substantial impact on the cleavage efficiency of the resulting agent, placing it among the worst analogues examined to date containing a single substituent removal.[37] One implication of these studies was the importance of the adoption of a compact, bent conformation productive for DNA cleavage, enlisting a turn at the threonine/valerate junction rather than adoption of an extended conformation characteristic of glycine. These studies complemented an earlier study of Hecht and co-workers where the substitution of $(gly)_n$, $n = 0-4$, for the L-threonine subunit caused no significant change in the cleavage selectivity although varying efficiencies of cleavage were noted.[50] Similarly, we have shown that N-methylation of the L-threonine linking amide provided an agent that was much less efficient (0.1−0.08×) at cleaving DNA and that its

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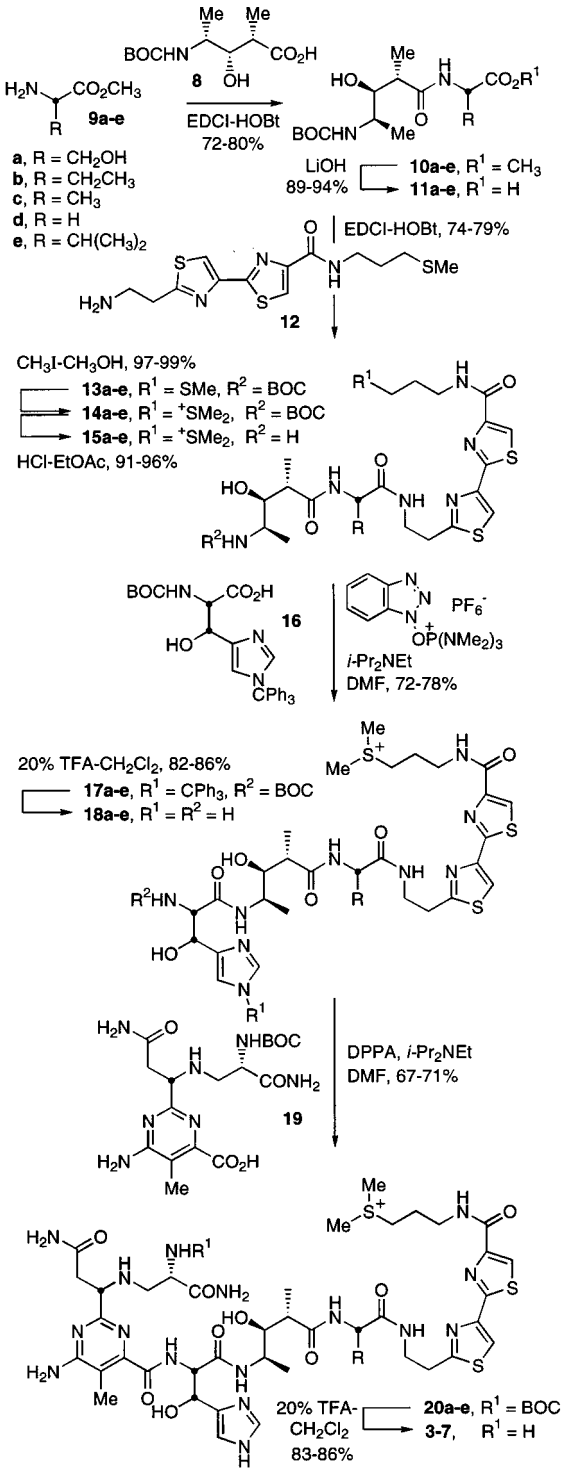
characteristic 5′-GC, 5′-GT cleavage selectivity was diminished, while the corresponding D-allo *N*-methyl threonine analogue failed to cleave DNA in a sequence specific manner.[38] Neither the glycine substitution nor the N-methylation of the L-threonine subunit substantially altered the metal chelation, H_2O_2 activation, catalytic efficiency, or inherent oxidation capabilities of the analogues. Rather, the studies suggest that the diminished DNA cleavage efficiency and selectivity may stem from a disruption of the deglycobleomycin A_2 interaction with duplex DNA and/or its reduced ability to adopt a productive bound conformation capable of C4′-H abstraction resulting in reduced DNA cleavage. The conformation of the linker of the metal-ligated bleomycin is especially interesting to probe, in light of the recent NMR structural models of Co(III)OOH and Zn bleomycin A_2.[51−53] Both studies highlight a turn in the acyclic peptide of the DNA-bound agent within the L-threonine subunit, implying that it may be induced by the L-threonine substitute. In addition, the NMR studies of the model Co(III)OOH complex of free and DNA-bound bleomycin revealed that the observed conformation may additionally be facilitated by a H-bond from the threonine NH to the proximal oxygen of the metal-bound hydroperoxide.[51,52] This H-bond could stabilize the productive bound conformation of the activated agent or fix the position or alignment of the reacting Fe−oxo intermediate for C4′ hydrogen abstraction. Additionally, it could stabilize the metal-bound hydroperoxide[54] or contribute to catalysis by assisting the chemistry of oxygen−oxygen bond cleavage in a fashion not yet understood. Finally, the threonine carbonyl of the DNA-bound conformation, but not of the free solution conformation, of Co(III)OOH bleomycin is positioned to accept a H-bond from the terminal oxygen of the hydroperoxide ligand. These related studies all highlight a potential important role for the L-threonine subunit of **1**−**2**, and the examination of **3**−**7** was anticipated to provide a detailed accounting of the role of the side chain substituents.

Synthesis of 3−7. We prepared the agents **3**−**7**, employing a recent modification[49] of our original synthesis of **2**[44] that was also utilized in our prior preparation of **6**.[37] The approach entails the synthesis of the pentapeptide S analogues **18a**−**e** and a final amide coupling at the *erythro*-β-hydroxy-L-histidine and pyrimidoblamic acid juncture rather than tetrapeptide S analogue (**15a**−**e**) coupling with the linked pyrimidoblamic acid/*erythro*-β-hydroxy-L-histidine. The latter coupling was found to suffer occasional dehydration with elimination of the β-hydroxy group of the L-histidine subunit upon carboxylate activation for amide bond coupling when conducted on relative large scales. This was not observed upon carboxylate activation of the *N*-BOC derivative of *erythro*-β-hydroxy-L-histidine presumably due to the lower acidity of its α-proton (*N*-BOC versus *N*-acyl derivative). Notably, this was accomplished with the sulfonium salt installed, on substrates incorporating only one protecting group, without deliberate protection of the imidazole, and without competitive imidazole acylation, provided that the coupling was effected by diphenylphosphoryl azide (DPPA). Coupling (2 equiv EDCI,[55] 1 equiv HOBt, DMF, 25 °C, 3 h, 72−80%) of L-Ser-OCH_3, methyl (*S*)-2-aminobutyrate, L-Ala-OCH_3, Gly-OCH_3, and L-Val-OCH_3 (**9a**−**e**) with (2*S*,3*S*,4*R*)-*N*-BOC-4-amino-3-hydroxy-2-methylpentanoic acid (**8**)[42] provided the dipeptides **10a**−**e** (Scheme 1). Sequential methyl ester

Scheme 1



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(55) EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; BOP reagent = benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; DCC = 1,3-dicyclohexylcarbodiimide.

Table 1. Summary of ΦX174 and w794 DNA Cleavage Properties

agent	relative efficiency of DNA cleavage ΦX174[a]	relative efficiency of DNA cleavage w794[b]	ratio of double to single strand cleavage[c]	DNA cleavage selectivity[b]
1, bleomycin A_2	2−5	5.8	1:6	5′-GC, 5′-GT > 5′-GA
2, deglycobleomycin A_2	1.0	1.0	1:12	5′-GC, 5′-GT > 5′-GA
3, R = CH_2OH	1−0.9	1.0	1:17	5′-GC, 5′-GT > 5′-GA
4, R = CH_2CH_3	0.50	0.45	1:21	5′-GC, 5′-GT > 5′-GA
5, R = CH_3	0.45	0.40	1:24	5′-GC, 5′-GT > 5′-GA
6, R = H	0.20[d]	0.25	1:31[d]	5′-GC, 5′-GT > 5′-GA
7, R = $CH(CH_3)_2$	0.17	0.20	1:25	5′-GC, 5′-GT > 5′-GA
Fe[a,b]	0.04	0.03	1:98	none

[a] Relative efficiency of supercoiled ΦX174 DNA cleavage, Fe(II)−O_2, 2-mercaptoethanol. The results are the average of six experiments. [b] Examined within [^{32}P]-5′-end-labeled w794, Fe(III)−H_2O_2. The results are the average of five experiments. [c] Ratio of double to single stranded cleavage of supercoiled ΦX174 DNA calculated as $F_{III} = n_2 \exp(-n_2)$, $F_I = \exp[-(n_1 + n_2)]$. [d] Previously reported as 0.20 and 1:29, ref 37.

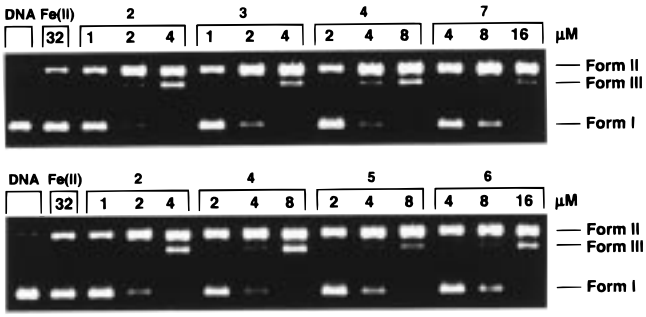


Figure 3. Agarose gel illustrating the cleavage reactions of supercoiled ΦX174 DNA by Fe(II)−agents at 25 °C for 1 h in buffer solutions containing 2-mercaptoethanol. After electrophoresis on a 1% agarose gel, the gel was stained with 0.1 μg/mL ethidium bromide and visualized on a UV transilluminator and quantified on a Millipore BioImage 60S RFLP system. The results are tabulated in Table 1.

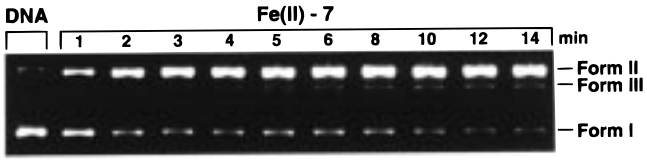


Figure 4. Representative kinetics of supercoiled ΦX174 DNA cleavage by Fe(II)−**7** (12 μM) in buffer solution containing 2-mercaptoethanol. The DNA cleavage reactions were run at 25 °C for various lengths of time, and electrophoresis was conducted on a 1% agarose gel. Direct fluorescence quantitation of the percentage of forms I−III DNA present at each time point was conducted using a Millipore BioImage 60S RFLP system visualized on a UV (312 nm) transilluminator in the presence of 0.1 μg/mL ethidium bromide taking into account the relative fluorescence intensities of forms I−III ΦX174 DNA (forms II and III have fluorescence intensities that are 0.7 times that of form I).

hydrolysis (2 equiv LiOH, *t*-BuOH/H_2O, 0 °C, 1 h, 89−94%), subsequent coupling of the resulting carboxylic acids **11a**−**e** with the bithiazole amine **12**[42] (2 equiv EDCI, 1 equiv HOBt, DMF, 25 °C, 5 h, 74−79%), and S-methylation (50 equiv CH_3I, CH_3OH, 25 °C, 24 h, 97−99%) of **13a**−**e** cleanly provided the *N*-BOC-tetrapeptide S analogues **14a**−**e**. Acid-catalyzed *N*-BOC deprotection (3.4 N HCl−EtOAc, 25 °C, 1.5 h) with liberation of the free amines **15a**−**e** (NH_4OH-CH_3OH, 25 °C, 1 h, 91−96%), subsequent coupling (1.5 equiv BOP reagent,[55] 2 equiv *i*-Pr_2NEt, DMF, 0−25 °C, 8 h, 72−78%) with N^{α}-BOC,N^{im}-CPh_3-*erythro*-β-hydroxy-L-histidine (**16**)[42] to provide **17a**−**e**, and acid-catalyzed deprotection (20% TFA-CH_2Cl_2, 0 °C, 2 h) with liberation of the free amines (NH_4OH-CH_3OH, 25 °C, 1 h) provided the pentapeptide S analogues **18a**−**e** in excellent conversions (82−86%). Their final couplings with N^{α}-BOC-pyrimidoblamic acid (**19**)[43] proceeded smoothly when effected by diphenylphosphoryl azide (DPPA, 1.5 equiv, 2 equiv *i*-Pr_2NEt, DMF, 0 °C, 10 h, 67−71%), and final acid-catalyzed deprotection of **20a**−**e** (20% TFA-CH_2Cl_2, 0 °C, 1.5 h) provided **3**−**7** (83−86%). When this last coupling reaction with **18d** was conducted with DCC[55] (2 equiv, 1 equiv HOBt, DMF, 0−25 °C, 10 h), the conversion to **20d** was much lower (29%).

DNA Cleavage Properties. Four assays were used to examine the DNA cleavage properties of **3**−**7**. The initial study of the relative efficiency of DNA cleavage was conducted with the Fe(II) complexes and supercoiled ΦX174 DNA in the presence of O_2 and 2-mercaptoethanol. Like Fe(II)−bleomycin A_2 and deglycobleomycin A_2, the Fe(II) complexes of all five agents produced single and double strand cleavage to afford relaxed (Form II) and linear (Form III) DNA, respectively (Figure 3 and Table 1). The replacement of threonine with serine gave **3** which was essentially indistinguishable from deglycobleomycin A_2. Thus, removal of the methyl group of the L-threonine side chain had little or no impact on the properties of **2**. The cleavage efficiencies of **4** and **5** were comparable although **4** was perceptibly better and both were less effective than **2** or **3** (0.45−0.5×). Thus, removal of the hydroxyl group of the L-threonine side chain reduced the cleavage efficiency 2-fold. Removal of the side chain altogether with **6** further and substantially reduced the cleavage efficiency (0.2× relative to **2**). Interestingly, replacement of the L-threonine subunit with valine in which the hydroxyl group is replaced with a methyl group led to an even greater 6-fold reduction in the efficiency of DNA cleavage.

The relative extent of double strand (ds) to single strand (ss) DNA cleavage was established in a study of the kinetics of supercoiled ΦX174 DNA cleavage to produce linear and circular DNA. We assumed a Poisson distribution for the formation of ss and ds breaks to calculate the average number of double and single strand cleavages per DNA molecule using the Freifelder−Trumbo equation.[56] The ratio of ds to ss cleavages observed with the Fe(II) complexes is illustrated in Figure 4 for **7**, and the full set of results is summarized in Table 1. The ratio of double to single strand DNA cleavage for **3** and **4** was established to be 1:17 and 1:21, that of **5** and **7** was 1:25, and that of **6** was 1:31. Consistent with the relative efficiencies of DNA cleavage, this was lower than bleomycin A_2 (1:6) or deglycobleomycin A_2 (1:12) but well above the ratio derived from uncomplexed Fe(II) cleavage (1:98).[57] With the significant exception of that for **6** (Gly), this diminishing trend in the ds/ss cleavage ratio parallels the trends observed in DNA-cleavage efficiency. For **6**, the ratio was 2−3× lower than that of **2**

(56) Freifelder, D.; Trumbo, B. *Biopolymers* **1969**, *7*, 681.

(57) A theoretical ratio of approximately 1:100 is required for the linear DNA to be the result of the random accumulation of single strand breaks within the 5386 base-pair size of ΦX174 DNA, assuming that sequential cleavage on the complementary strands within 15 base-pairs is required for formation of linear DNA.

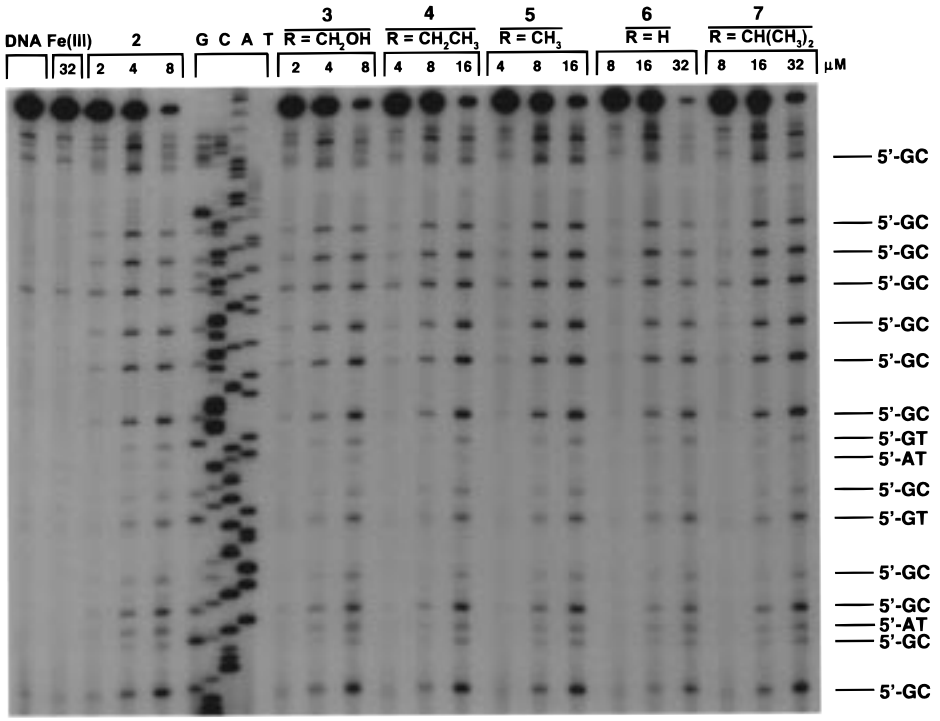


Figure 5. Cleavage of double-stranded DNA by Fe(III)−agent (SV40 DNA fragment, 144 base pairs, nucleotide no. 5238-138, clone w794) in phosphate/KCl buffer containing H_2O_2. The DNA cleavage reactions were run for 30 min at 37 °C, and electrophoresis was run on an 8% denaturing PAGE and visualized by autoradiography.

while the remaining agents were <2× less effective, suggesting that **6** is uniquely less prone to promote ds cleavage as suggested in prior studies.[37]

The assessment of the selectivity of DNA cleavage along with an additional assessment of the relative efficiency of DNA cleavage were carried out with duplex w794 DNA[58] by monitoring strand cleavage of singly [^{32}P]-5′-end-labeled double-stranded DNA by the Fe(III)−complexes upon activation with H_2O_2[59] in 10 mM phosphate buffer (pH 7.0). This protocol has proven more sensitive to the distinctions in the relative efficiency of DNA cleavage than the ΦX174 supercoiled DNA cleavage assay, but both have always provided the same trends in our hands. Thus, incubation of the labeled duplex DNA with the agents in the presence of equimolar $FeCl_3$ and excess H_2O_2 led to DNA cleavage. Because of the poor efficiency of DNA cleavage exhibited by **6** in our earlier study (**6** = 0.04×, Fe(III) = 0.006×), the assay was modified by reducing the amount of end-labeled DNA employed. The net result was that the agent concentration range over which cleavage occurred was smaller and the absolute concentration required for DNA cleavage was reduced but the cleavage selectivity was more readily distinguishable. Thus, the magnitude of the relative efficiency differences was reduced, but the relative trend was not altered. For example, **6** was found to be 25× less effective than **2** but 7× more effective than Fe(III) itself in our earlier study,[37] while in the present assay it was 4× less effective than **2** and 8× more effective than Fe(III). Similarly, deglycobleomycin A_2 was 167× more effective than Fe(III) in our prior studies but 33× more effective under the conditions employed in the present assay. With this 5-fold compression of the dynamic range (167×/33× = 5), the 4-fold reduction in cleavage efficiency observed herein with **6** would appear to be consistent with our prior observations (25×/4× = 6.25).[37]

```
5'   CGAATTCTGCGGTACCTTTCCCA A
                              A
3'   GCTTAAGACGCCATGGGAAAGGGA A
                    *
```

Figure 6. Internally labeled hairpin oligonucleotide containing GTAC hot spot for ds cleavage. ^{32}P-labeling site is marked by ∗, ss cleavage sites are underlined, and ds cleavage site is in bold.

Typical comparisons of **3**−**7** are illustrated in Figure 5. Under all conditions examined, **3**−**7** were found to cleave w794 double-stranded DNA with the characteristic 5′-GT, 5′-GC > 5′-GA sequence selectivity of bleomycin A_2 and deglycobleomycin A_2 (**2**). Consistent with the ΦX174 DNA studies, **3** proved to be the most efficient analogue and was essentially indistinguishable from deglycobleomycin A_2. The analogues **4** and **5** were progressively less efficient, and **6** and **7** were even less efficient (Table 1). Thus, removal of the L-threonine hydroxyl group or its replacement with a methyl group significantly reduced the cleavage efficiency, while the removal of the methyl group had no impact. A further significant reduction in cleavage efficiency was observed when the side chain was removed altogether.

In a fourth assessment of the DNA cleavage properties, **6** was examined in a hairpin cleavage assay that enlists an internally labeled site to establish the relative ss and ds cleavage (Figure 6).[60] For bleomycin A_2, the ds to ss cleavage ratio at the T13 + T38 ds cleavage site is 1:3 under single turnover

(58) Ambrose, C.; Rajadhyaksha, A.; Lowman, H.; Bina, M. *J. Mol. Biol.* **1989**, *209*, 255. Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.

(59) Natrajan, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 3997. Natrajan, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 4532. Hamamichi, N.; Natrajan, A.; Hecht, S. M. *J. Am. Chem. Soc.* **1992**, *114*, 6278.

(60) (a) Absalon, M. J.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1995**, *34*, 2065. (b) The larger increases in the magnitude of differences observed with the hairpin assay and, to a lesser extent, with the w794 DNA versus the ΦX174 DNA can be attributed in part to the corresponding amount of unlabeled carrier DNA which competes for consumption of agent and its oxidizing capability. Additionally, the hairpin assay is run under single turnover conditions which may affect the magnitude of the differences.

Table 2. Styrene Oxidation[a]

agent	Styrene epoxide (mM)	phenylacetaldehyde (mM)	ratio	total product (mM)	rel efficiency
Fe(III)−**2**	1.79	0.73	2.45	2.52	1.0
Fe(III)−**6**	0.87	0.40	2.18	1.27	0.50
Fe(III)	0	0	0	0	0
H_2O_2	0	0	0	0	0
Fe(III)−H_2O_2	0	0	0	0	0
2	0	0	0	0	0
2−H_2O_2	0	0	0	0	0
Fe(III)−**2**[b]	0	0	0	0	0

[a] 500 μM (Fe(III)−agent, 30 mM H_2O_2, 50 mM styrene, 0 °C, 1.5 h, 80% CH_3OH-H_2O. Results reported are the average of two runs. [b] 500 μM Fe(III)−**2** under identical conditions without H_2O_2.

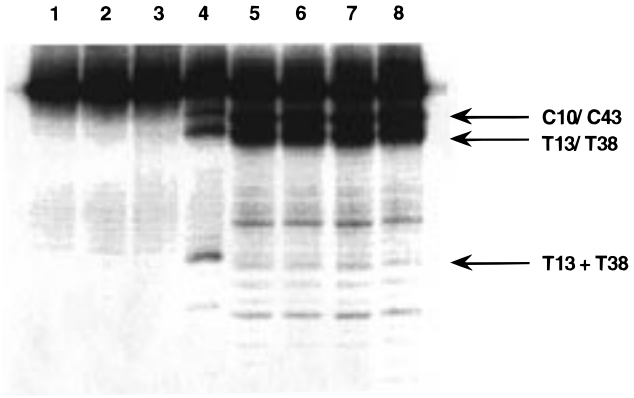


Figure 7. Cleavage of hairpin oligonucleotide by **6** activated with Fe(II)−O_2. Lane 1, hairpin only; lane 2, hairpin + Fe(II); lane 3, hairpin + **6**, no Fe(II); lane 4, 20 μM bleomycin A_2; lane 5, 10 s digestion with 300 μM **6**; lane 6, 30 s digestion with 300 μM **6**; lane 7, 60 s digestion with 300 μM **6**; lane 8, 120 s digestion with 300 μM **6**.

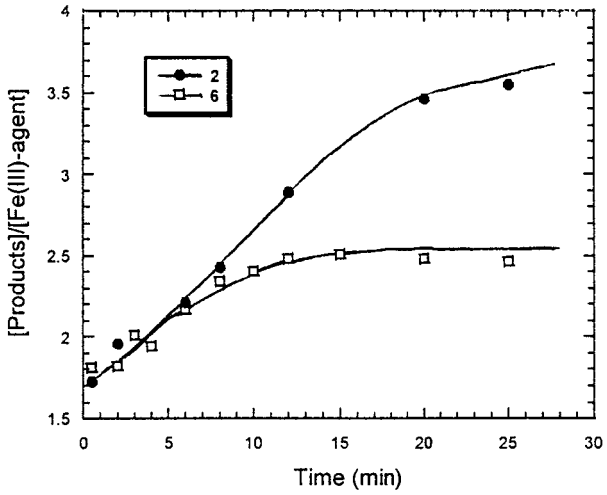


Figure 8. Kinetics of styrene (50 mM, 30 mM H_2O_2) oxidation catalyzed by the Fe(III) complex of agent **2** and **6** (500 μM). The ratio of total concentration of products (styrene epoxide and phenylacetaldehyde) versus the initial concentration of Fe(III)−agent complex (turnover) is plotted against time.

conditions.[60] In these studies, **6** was activated with either Fe(III)/H_2O_2 or with Fe(II)/O_2 in the absence of external reducing equivalents. The latter activation gives a good indication of the ds versus ss cleavage ratio since it ensures single turnover conditions. As can be seen in Figure 7, the ds cleavage band is significantly less pronounced for **6** than it is for **1** with a ratio of ds to ss cleavage at the T13 + T38 site of approximately 1:20. Thus, the ability of **6** to promote ds cleavage is greatly impaired but not completely suppressed. Its DNA cleavage selectivity (5′-GC/5′-GT) as well as its reduced efficiency (0.03−0.04× deglycobleomycin A_2) in the hairpin assay were in accord with the results obtained in the ΦX174 and w794 DNA-cleavage assays.[60b]

Oxidation of Styrene. Relative Kinetics versus Efficiencies of Oxidation. In our final efforts to characterize the differences in the properties of the agents, the ability of the Fe(III) complexes of **6** and deglycobleomycin A_2 (**2**) to mediate the oxidation of styrene were compared.[59] The oxidation produces both styrene epoxide and phenylacetaldehyde and a solution of 500 μM Fe(III)−**2**, 50 mM styrene (excess), and 30 mM H_2O_2 (0 °C, 1.5 h) produced 1.79 mM styrene epoxide and 0.73 mM phenylacetaldehyde constituting five oxidations for each Fe(III)−**2** (Table 2). Under identical conditions, the glycine-containing agent produced 0.87 mM styrene epoxide and 0.40 mM phenylacetaldehyde constituting 2.5 oxidations for each Fe(III)−**6**. Thus, the same products and product distribution were observed, but **6** proved to be only half as efficient as **2**.

The kinetics of the styrene oxidation were examined in efforts to establish whether this distinction was derived from different rates of oxidation or different catalytic efficiencies of the two agents. Under the pseudo-first-order oxidation conditions of excess styrene and H_2O_2, the oxidation rates of **2** and **6** within the first 5 min were not distinguishable (Figure 8). A full oxidation turnover occurred in less than 30 s, the first time point in the kinetic measurements, and at that point, the two agents both had oxidized 1.74−1.80 equiv of styrene. Over the next 5 min, the two agents exhibited essentially indistinguishable kinetics of oxidation, most likely reflecting the reactivation versus oxidation kinetics. After 5−10 min, the rates of oxidation rates diverged, slowed, and the rate of **6** decreased more rapidly than that of **2**. Ultimately the oxidation halted at 15 min for **6** and 45 min for **2**, constituting 2.52 and 5.04 oxidations for **6** and **2**, respectively, indicating the exhaustion of the Fe(III)−agents. Although the genesis of the initial oxidation burst and the initial oxidation kinetics of **2** and **6** were not determined, the studies did allow us to establish that differences in the styrene oxidation efficiencies of **2** and **6** may be attributed to differences in total turnover and not to the relative rates of oxidation. Since these conditions parallel those enlisted in the w794 DNA cleavage studies, the results imply that part of the efficiency differences observed with **6** and **2** may be attributed to their relative stabilities and number of catalyst turnovers and is unlikely due to differences in steady-state oxidation kinetics. However, the effect is modest and insufficient to account for the extent of the differences in **2** and **6**.

A similar set of observations were made in a study of the *N*-methyl threonine analogue of deglycobleomycin A_2.[38] The DNA cleavage efficiency of the Fe(III) complex was substantially diminished (0.08−0.1×) but the inherent styrene oxidation capability of the agent was relatively unaffected (0.52×) and similar to that observed with **6**. These results and those of **6**

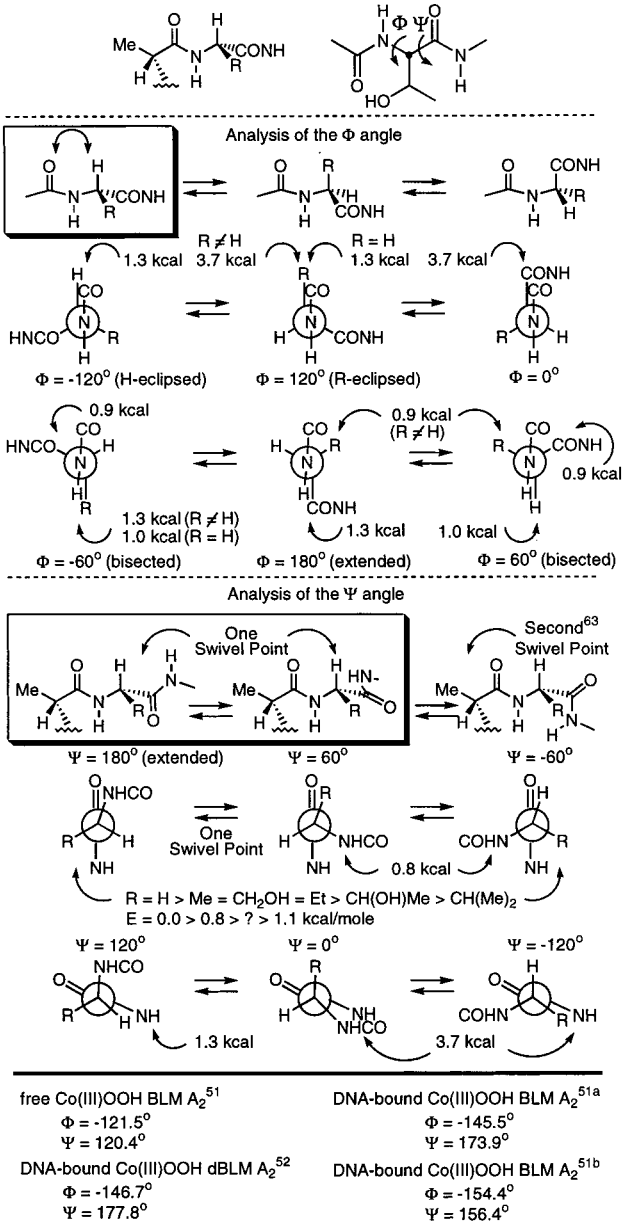


Figure 9. Analysis of the Φ and Ψ angles of the threonine subunit.[62]

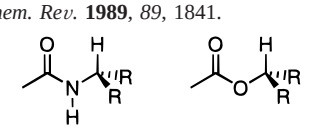
detailed herein suggest that any H-bonding interaction of the threonine subunit with a metal-bound hydroperoxide or subsequent activated species does not appear to be contributing to the catalysis of the oxidation reaction but rather may be contributing to the inherent stability of the complex, providing an improvement in the number of oxidation turnovers.

Correlation with Conformational Effects of the Substitutions. The results of the DNA cleavage comparisons proved consistent with the conformational effects of the substituent within the linker region. The most dominant effect of the substitutions resides with the amide bond linking the valerate and threonine subunits. When the L-threonine subunit bears a substituent (Figure 9, R ≠ H), the H-eclipsed conformation (Φ = −120°) can be expected to dominate, on the basis of the well-established conformational preference for amides and esters of cyclohexylamines or alcohols.[61] This conformation is observed in the refined solution conformation of Co(III)OOH bleomycin A_2 (Φ = −121.5°, Figure 10a)[51] and its DNA-bound conformation (Φ = −145.5°, Figure 10b)[51] as well as in the DNA-bound conformation of Zn bleomycin A_2[53] and constitutes one site at which a bend in the structure is found (Φ dihedral angle ca. −120°). This facilitates the adoption of a compact conformation implicated in earlier studies.[37,44] Removal of the L-threonine side chain (R = H) provides two equivalent and equally accessible H-eclipsed conformations (Φ = 120° and −120°), of which only one would be productive for DNA binding and cleavage, and one fully extended conformation (Φ = 180°) of an equal energy (Figure 9). It also reduces the energy difference between these three conformations and the remaining two bisected conformations (Φ = 60° and −60°) to ca. 0.6 kcal/mol. The consequences are that the adoption of the single conformation implicated in DNA cleavage (Φ ≅ −120°) out of these accessible five is considerably less favorable when R = H. This is consistent with the observation that removal of the L-threonine side chain has a substantial effect on the cleavage efficiency (w794, 0.2−0.04×).

The substituent has a less pronounced and more subtle effect on the conformation within the L-threonine subunit itself (Ψ dihedral angle). When substituted, two accessible carbonyl-eclipsed conformations are of comparable energy (Figure 9, Ψ = 180° and 60°). The former is observed in the DNA-bound conformation of Co(III)OOH bleomycin A_2 (Ψ = 173.9°) while an average intermediate bisected conformation is observed free in solution (Ψ = 120.4°).[51] As the size of this substituent increases, the productive NHCO eclipsed conformation (Ψ = 180°) would be expected to become increasingly disfavored. It may be that the diminished DNA cleavage efficiency within the series (R = CH_3 ≅ Et > *i*-Pr) reflects this increasing difficulty in accessing the conformation (Ψ = 180°), observed with the DNA-bound agent. The exceptions to this generalization, R = $CH(OH)CH_3$ and CH_2OH, can enlist the side-chain hydroxyls to adopt intramolecular backbone carbonyl H-bonded conformations to restabilize the extended conformation (Figure 11).[64] This is especially true of the six-membered ring H-bond to the threonine carbonyl which is observed in the free solution conformation (Figure 11). However, close examination of the model Co(III)OOH bleomycin A_2-bound conformation suggests an additional and provocative explanation for the behavior of the agents containing a side-chain hydroxyl group. Although neither the model of DNA-bound Co(III)OOH BLM A_2 nor the Hecht model of DNA-bound Zn BLM A_2 reveals a potential interaction of the hydroxyl group with DNA itself, the primary amide carbonyl of the pyrimidoblamic acid C2 acetamido side chain lies within H-bonding distance (1.70 Å, O··H−O angle = 147°) of the threonine side-chain hydroxyl group in the Stubbe model (Figure 10b). The adoption of this H-bond releases the H-bond to the threonine carbonyl allowing the adoption of the extended (Ψ = 180°) conformation and aligns the threonine carbonyl to accept a H-bond from the peroxide ligand of Co(III)OOH bleomycin A_2 (see Figure 10, 2.24 → 1.87 Å, O−H- - -O angle 130.7 → 162.7°). This provides a tightly organized H-bonded metal complex that also includes the additional threonine NH H-bond to the proximal oxygen of

(61) Hoffman, R. W. *Chem. Rev.* **1989**, *89*, 1841.

preferred conformations



(62) H/H, H/R, and R/R 1,2-eclipsing interactions were estimated using H/H (1.0 kcal/mol), H/CH_3 (1.3 kcal/mol), and CH_3/CH_3 (3.7 kcal/mol). Gauche interactions were estimated, enlisting $^1/_2$ the known *A*-values.

(63) Boger, D. L.; Ramsey, T. M.; Cai, H.; Hoehn, S. T.; Stubbe, J. *J. Am. Chem. Soc.* **1998**, *120*, 9149.

(64) Ser H-bonding: Siemion, I. Z.; Picur, B. *Biophys. Chem.* **1988**, *31*, 71. Lipkind, G. M.; Arkhipova, S. F.; Popov, E. M. *Int. J. Pept. Res.* **1973**, *5*, 381. Marcus, E.; Keller, D. A.; Shibota, M.; Ornstein, R. L.; Rein, R. *Chem. Phys.* **1996**, *204*, 157.

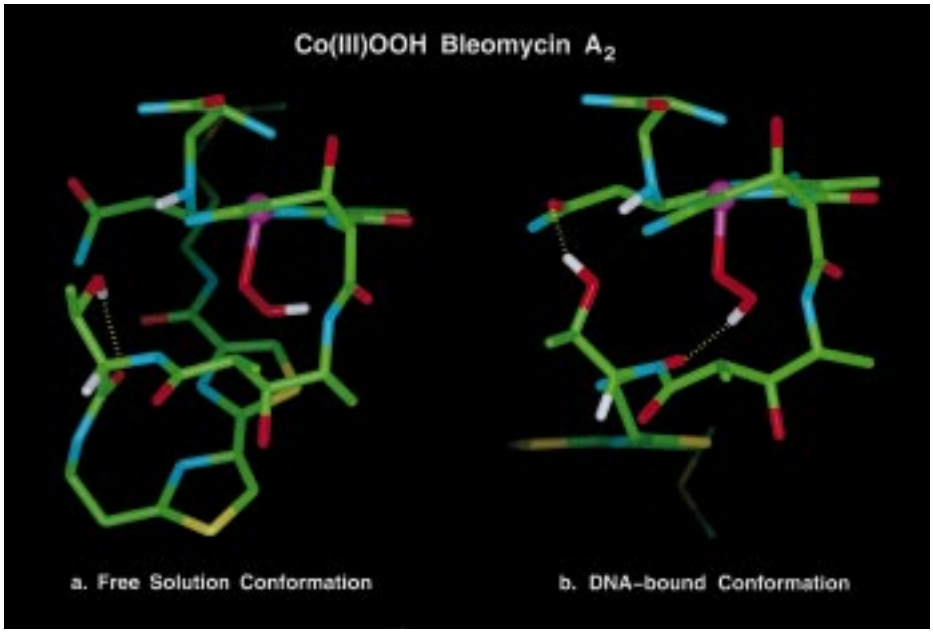


Figure 10. Views of the (a) free and (b) DNA-bound conformation[51a] of Co(III)OOH bleomycin A_2, highlighting the compact conformations, the L-threonine subunit conformations, and the entire rigid, compact linker domain.

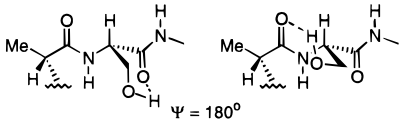


Figure 11.

the peroxide ligand. It is plausible that the acetamido side chain CH_2CONH_2 (4×)[44] and serine/threonine OH (2×) contribution to the DNA cleavage efficiency rests with these intramolecular H-bonding interactions that could stabilize or induce the adoption of the compact conformation implicated in productive DNA cleavage.

High level ab initio computational studies of NAc-Gly-NHMe, NAc-Ala-NHMe, NAc-Val-NHMe, and NAc-Ser-NHMe (Figure 12)[65] support the conclusions drawn from the qualitative analysis illustrated in Figure 9. NAc-Gly-NHMe may adopt two equivalent low energy perpendicular conformations ($\Phi = -85.4°, +84.5°$) which represent opposite turns in the peptide, a readily accessible extended conformation ($\Phi = 180°$, $\Delta E = 0.8$ kcal/mol), and two $\Phi = -162°$ conformations ($\Delta E = 1.5$ kcal/mol). In contrast, Ala and Val are restricted to a single NHCO perpendicular conformation ($\Phi = -84.6$, ΔE for $74.6° = 2.6$ kcal/mol for Ala) and a related $\Phi = -157.3°$ conformation ($\Delta E = 0.4$ kcal/mol for Ala). Both induce a common turn direction ($\Phi = -85°$ to $-157°$), possess comparable energies (a near flat barrier to interconversion), and approximate the conformations found for free ($\Phi = -121°$) and DNA-bound Co(III)OOH bleomycin A_2 ($\Phi = -145°$).[51]

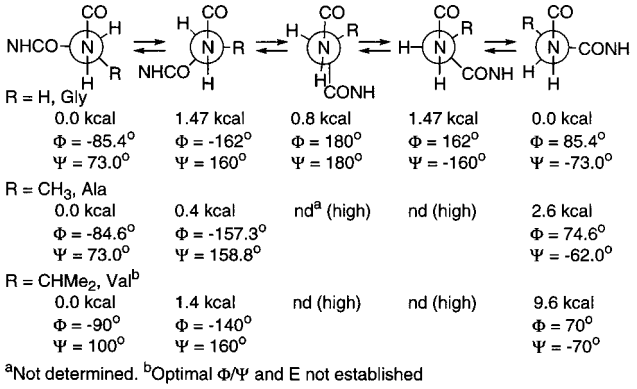


Figure 12. Ab initio calculations.[65]

Moreover, the $\Psi \cong 180°$ conformational isomer implicated in DNA cleavage becomes increasingly disfavored as the size of the substituent increases.

Conformational energy calculations of NAc-X-NHMe (X = amino acid) and calculated Boltzmann probabilities (300 K, Table 3) for the 20 naturally occurring amino acids and 2-aminobutyric acid have been disclosed.[66] In addition to defining the characteristic $\Phi \cong -120°$, $\Psi = 60-180°$ conformational space for the amino acids discussed, the DNA cleavage efficiencies detailed herein correlate remarkably well with the probability for adoption of the solution phase conformation of Co(III)OOH bleomycin A_2 ($\Psi = 120.4°$), do not appear to correlate with the probability of adoption of the DNA-bound conformation ($\Psi = 173.9°$), and with the exception of Val, correlate with the total distribution within these two related conformations (Table 3). The simplest interpretation of this correlation is that the L-threonine substituents facilitate preorganization of bleomycin A_2 into a conformation that ultimately leads to productive DNA cleavage.

In our own modeling studies (OPLS Amber, H_2O), we have also observed a subtle interplay between the Ψ conformational isomers (Ψ = ca. 60° or 180°) which influences the Φ dihedral

(65) NAc-Gly-NHMe: Klimkowski, V. J.; Schafer, L.; Momany, F. A.; Alsenoy, C. V. *J. Mol. Struct. (THEOCHEM)* **1985**, *124*, 143. Schafer, L.; Alsenoy, C. V.; Scarsdale, J. N. *J. Chem. Phys.* **1982**, *76*, 1439. Scarsdale, J. N.; Alsenoy, C. V.; Klimkowski, V. J.; Momany, F. A. *J. Am. Chem. Soc.* **1983**, *105*, 3438. NAc-Ala-NHMe: Schafer, L.; Klimkowski, V. J.; Momany, F. A.; Chuman, H.; Alsenoy, C. V. *Biopolymers* **1984**, *23*, 2335. Schafer, L.; Bin Drees, I. S.; Frey, R. F.; Alsenoy, C. V.; Ewbank, J. D. *J. Mol. Struct. (THEOCHEM)* **1995**, *338*, 71. Mohle, K. R.; Hofmann, H.-J. *J. Mol. Struct. (THEOCHEM)* **1993**, *285*, 211. NAc-Ser-NHMe: Siam, K.; Kimkowski, V. J.; Alsenoy, C. V.; Ewbank, J. D.; Schafer, L. *J. Mol. Struct. (THEOCHEM)* **1987**, *152*, 261. Siam, K.; Kulp, S. Q.; Ewbank, J. D.; Schafer, L. *J. Mol. Struct. (THEOCHEM)* **1989**, *184*, 143. For NAc-Val-NHMe: Paterson, Y.; Leach, S. J. *Macromolecules* **1978**, *11*, 409. See also: Kollman, P. A.; Gould, I. R. *J. Phys. Chem.* **1992**, *96*, 9255. Gould, I. R.; Cornell, W. D.; Hillier, I. H. *J. Am. Chem. Soc.* **1994**, *116*, 9250.

(66) Vasquez, M.; Nemethy, G.; Scheraga, H. *Macromolecules* **1983**, *16*, 1043. Zimmerman, S. S.; Pottle, M. S.; Nemethy, G.; Scheraga, H. A. *Macromolecules* **1977**, *10*, 1.

Table 3.

agent	rel cleavage efficiency (ΦX174)	Boltzmann prob.[a] Ψ = 20 to 130°	Boltzmann prob. Ψ = 130 to −140°	total Boltzmann prob.
Thr, R = $CH(OH)CH_3$	1.0	0.798	0.153	0.951
Ser, R = CH_2OH	1−0.9	0.776	0.087	0.863
Abu, R = Et	0.50	0.605	0.191	0.796
Ala, R = CH_3	0.45	0.589	0.138	0.727
Gly, R = H	0.20	0.425	0.049	0.474
Val, R = *i*-Pr	0.17	0.390	0.335	0.725

[a] Taken from ref 66, for AcN-X-NHMe. Free Co(III)OOH BLM A_2 (Φ = −121.5°, Ψ = 120.4°). DNA-bound Co(III)OOH BLM A_2 (Φ = −145.5°, Ψ = 173.9°).

angle. As the R substituent increases in size, the Ψ ca. = 180° conformation becomes increasingly disfavored, diminishing the capabilities for adoption of the conformation implicated in DNA cleavage. The exceptions to this are serine and threonine which provide compensating stabilization of the conformation through H-bonding. The difference in energy is small for the two accessible Ψ conformational isomers, and both are only slightly more stable than a third, bisected conformation (Ψ = 120°) that lies between the two. In fact, the structure for both free Co(III)OOH bleomycin A_2 as well as the DNA-bound conformation of Zn bleomycin A_2[53] are represented as this bisected conformation (Ψ = 120°). Thus, the local threonine conformation is characterized by a Φ = −120, Ψ = 60−180° accessible conformational space. It is thus tempting to suggest that this site also constitutes one of the critical swivel points for the agent that permits the adoption of a class of related bound conformations (Ψ = 60−180°) adaptable to variable conformational characteristics required of the multiple cleavage sites, including both primary cleavage and subsequent ds cleavage on the complementary strand from a common intercalation site.

Conclusions

Variations in the L-threonine subunit substituents had no impact on the characteristic 5′-GC, 5′-GT cleavage selectivity of deglycobleomycin but did have a large effect on the cleavage efficiency. The studies define a clear potentiating role for the L-threonine hydroxyl group, highlight the additional importance of the presence of a substituent on the L-threonine subunit (R ≠ H), and suggest the effect is unusually sensitive to the size of the substituent (R = CH_3 ≅ Et > *i*-Pr). A good correlation was observed between the cleavage efficiencies and the calculated Boltzmann probabilities[66] of adopting the L-threonine subunit local conformation observed with free Co(III)OOH bleomycin A_2 and, with the exception of Val, with the total distribution within the free and DNA-bound conformations of Co(III)OOH bleomycin A_2. This suggests that the L-threonine side chain facilitates preorganization of bleomycin A_2 into a conformation that leads to productive DNA cleavage. The important role of the L-threonine hydroxyl group may be attributed to either intramolecular H-bonding to the threonine carbonyl or, more provocatively, to the distal pyrimidoblamic acid C2 acetamido side-chain carbonyl and the metal-coordinated, β-amino group of the C2 side chain, stabilizing a preorganized conformation of activated bleomycin productive for DNA cleavage. However, we cannot rule out an intermolecular H-bond with duplex DNA itself although structural models to date have not provided evidence for such an interaction. The additional, simpler effect of the substituent presence is consistent with a subtle but important role in restricting the available conformations accessible to the agent. The presence of a substituent (R ≠ H) restricts the valerate−threonine amide to a single orientation (Φ ≅ −120°), inducing a turn in the linker domain characteristic of the compact bound conformation observed in free Co(III)OOH bleomycin A_2 and both models of DNA-bound bleomycin A_2.[51−53] Removal of this substituent (R = H) results in five accessible conformations, at least three of which may be nonproductive for DNA cleavage. Clearly, from the relative behavior of **6**, the extended conformation characteristic of Gly is not optimal for DNA cleavage. In addition, as the size of this substituent increases, the DNA-cleavage efficiency decreases (R = Me ≅ Et > *i*-Pr), consistent with the observation that the conformations nonproductive for primary strand cleavage becomes increasingly favored. Both of these observations are consistent with the premise that linker region substituents preorganize the agent, facilitating adoption of the productive DNA-bound conformation that enhances the cleavage selectivity over nonproductive background cleavage and increases the overall cleavage efficiency.

Experimental Section[67]

***N*$^{\beta}$-[1-Amino-3(*S*)-[4-amino-6-[[[1(*S*)-(((4(*S*)-(((1(*S*)-(((2-(4′-((((3-dimethylsulfonio)-1-propyl)amino)carbonyl)-2′,4-bithiazol-2-yl)-1-ethyl)amino)carbonyl)-2-hydroxy-1-ethyl)amino)carbonyl)-3(*S*)-hydroxy-2(*R*)-pentyl)amino)carbonyl)-2(*R*)-hydroxy-2-(imidazol-4-yl)-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-β-aminoalanine Amide (3).**[67] A solution of **20a**[67] (5 mg, 0.005 mmol) in CH_2Cl_2 (100 μL) at 0 °C was treated with 20% TFA−CH_2Cl_2 (200 μL) and the mixture was stirred at 0 °C (1.5 h) before the solvent was evaporated in vacuo. Reverse phase chromatography (C-18, 1.2 × 2.0 cm, 0−60% CH_3OH−H_2O gradient elution) afforded **3** (3.8 mg, 4.6 mg theoretical, 85%) as a white film: R_f = 0.2 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{25}_D$ −8.0 (*c* 0.10, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.19 (s, 1H), 8.11 (s, 1H), 7.68 (br s, 1H), 7.10 (br s, 1H), 5.17 (d, *J* = 6.5 Hz, 1H), 4.79 (d, *J* = 6.5 Hz, 1H), 4.41 (dd, *J* = 5.5, 5.5 Hz, 1H), 3.97 (dd, *J* = 5.5, 9.0 Hz, 1H), 3.91 (dd, *J* = 5.5, 5.5 Hz, 1H), 3.78 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.74 (dd, *J* = 5.5, 5.5 Hz, 2H), 3.65 (m, 4H), 3.57 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.35 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.26 (dd, *J* = 7.0, 7.0 Hz, 2H), 2.91 (s, 6H), 2.86 (m, 2H), 2.71 (dd, *J* = 4.5, 15.0 Hz, 1H), 2.53 (dd, *J* = 9.0, 15.0 Hz, 1H), 2.41 (dq, *J* = 7.0, 7.0 Hz, 1H), 2.14 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.11 (d, *J* = 6.5 Hz, 3H), 1.08 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3282, 2912, 1656, 1641, 1589, 1548, 1487, 1364, 1241, 1205, 1092 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1033.3975 (M^+, $C_{41}H_{61}N_{16}O_{10}S_3$ requires 1033.3919).

***N*$^{\beta}$-[1-Amino-3(*S*)-[4-amino-6-[[[1(*S*)-(((4(*S*)-(((1(*S*)-(((2-(4′-((((3-dimethylsulfonio)-1-propyl)amino)carbonyl)-2′,4-bithiazol-2-yl)-1-ethyl)amino)carbonyl)-1-propyl)amino)carbonyl)-3(*S*)-hydroxy-2(*R*)-pentyl)amino)carbonyl)-2(*R*)-hydroxy-2-(imidazol-4-yl)-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-β-aminoalanine amide (4):**[67] (86%) R_f = 0.2 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{25}_D$ −6.0 (*c* 0.05, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.19 (s, 1H), 8.10 (s, 1H), 7.67 (br s, 1H), 7.11 (br s, 1H), 5.13 (d, *J* = 6.5 Hz, 1H), 4.76 (d, *J* = 6.5 Hz, 1H), 4.23 (dd, *J* = 6.0, 8.0 Hz, 1H), 3.96 (dd, *J* = 4.5, 9.5 Hz, 1H), 3.91 (dd, *J* = 5.0, 6.5 Hz, 1H), 3.76 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.69 (dd, *J* = 7.0, 7.0 Hz, 1H), 3.61 (m, 3H), 3.57 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.35 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.27 (dd, *J* = 7.0, 7.0 Hz,

(67) Full characterization of intermediates **10a**−**e**, **11a**−**e**, **13a**−**e**, **14a**−**e**, **15a**−**e**, **17a**−**e**, **18a**−**e**, and **20a**−**e** is provided in Supporting Information.

2H), 2.92 (s, 6H), 2.85 (d, J = 9.0 Hz, 2H), 2.71 (dd, J = 4.5, 15.0 Hz, 1H), 2.52 (dd, J = 9.0, 15.0 Hz, 1H), 2.44 (dq, J = 7.0, 7.0 Hz, 1H), 2.27 (s, 3H), 2.14 (tt, J = 7.0, 7.0 Hz, 2H), 1.75 (m, 1H), 1.61 (m, 1H), 1.12 (d, J = 7.0 Hz, 3H), 1.07 (d, J = 7.0 Hz, 3H), 0.88 (dd, J = 7.5, 7.5 Hz, 3H); IR (neat) ν_{max} 3292, 2933, 1656, 1641, 1589, 1482, 1241, 1205, 1092 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1031.4182 (M^+, $C_{42}H_{63}N_{16}O_9S_3$ requires 1031.4126).

***N*$^{\beta}$-[1-Amino-3(*S*)-[4-amino-6-[[[1(*S*)-(((4(*S*)-(((1(*S*)-(((2-(4′-((((3-dimethylsulfonio)-1-propyl)amino)carbonyl)-2′,4-bithiazol-2-yl)-1-ethyl)amino)carbonyl)-1-ethyl)amino)carbonyl)-3(*S*)-hydroxy-2(*R*)-pentyl)amino)carbonyl)-2(*R*)-hydroxy-2-(imidazol-4-yl)-1-ethyl]-amino]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-*β*-aminoalanine amide (5):**[67] (85%) R_f = 0.2 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{25}_D$ −7.5 (*c* 0.07, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.19 (s, 1H), 8.11 (s, 1H), 7.67 (br s, 1H), 7.11 (br s, 1H), 5.11 (d, J = 6.5 Hz, 1H), 4.76 (d, J = 6.5 Hz, 1H), 4.33 (dd, J = 7.0, 7.0 Hz, 1H), 3.96 (dd, J = 5.5, 9.0 Hz, 1H), 3.91 (dd, J = 12.0, 5.5 Hz, 1H), 3.75 (dd, J = 6.0, 6.0 Hz, 1H), 3.66 (dd, J = 7.0, 7.0 Hz, 1H), 3.61 (m, 4H), 3.58 (dd, J = 6.0, 6.0 Hz, 2H), 3.37 (dd, J = 6.5, 6.5 Hz, 2H), 3.27 (dd, J = 7.0, 7.0 Hz, 2H), 2.92 (s, 6H), 2.85 (m, 2H), 2.70 (dd, J = 4.5, 15.0 Hz, 1H), 2.52 (dd, J = 9.0, 15.0 Hz, 1H), 2.39 (dq, J = 7.0, 7.0 Hz, 1H), 2.27 (s, 3H), 2.15 (tt, J = 2.0, 7.0 Hz, 2H), 1.30 (d, J = 7.0 Hz, 3H), 1.10 (d, J = 7.0 Hz, 3H), 1.08 (d, J = 7.0 Hz, 3H); IR (neat) ν_{max} 3333, 2923, 1656, 1635, 1589, 1482, 1241, 1205, 1087 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1017.3607 (M^+, $C_{41}H_{61}N_{16}O_9S_3$ requires 1017.3969).

***N*$^{\beta}$-[1-Amino-3(*S*)-[4-amino-6-[[[1(*S*)-(((4(*S*)-(((1-(((2-(4′-((((3-dimethylsulfonio)-1-propyl)amino)carbonyl)-2′,4-bithiazol-2-yl)-1-ethyl)amino)carbonyl)methyl)amino)carbonyl)-3(*S*)-hydroxy-2(*R*)-pentyl)amino)carbonyl)-2(*R*)-hydroxy-2-(imidazol-4-yl)-1-ethyl]-amino]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-*β*-aminoalanine (6):**[67] (83%) R_f = 0.10 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{23}_D$ +14 (*c* 0.04, 0.1 N aqueous HCl); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.20 (s, 1H), 8.11 (s, 1H), 7.77 (s, 1H), 7.13 (s, 1H), 5.11 (d, J = 6.6 Hz, 1H), 4.77 (d, J = 6.6 Hz, 1H), 4.02 (m, 2H), 3.93 (m, 2H), 3.64 (m, 4H), 3.60 (m, 3H), 3.37 (dd, J = 7.5, 7.5 Hz, 2H), 3.26 (dd, J = 6.6, 6.6 Hz, 2H), 3.00 (m, 2H), 2.93 (s, 6H), 2.75 (m, 1H), 2.53 (m, 1H), 2.38 (dd, J = 6.8, 6.8 Hz, 1H), 2.26 (s, 3H), 2.15 (tt, J = 6.8, 6.8 Hz, 2H), 1.12 (d, J = 6.8 Hz, 3H), 1.11 (d, J = 6.7 Hz, 3H); ^{1}H NMR (D_2O, 400 MHz) δ 7.99 (s, 1H), 7.87 (s, 1H), 7.73 (s, 1H), 7.17 (s, 1H), 5.10 (m, 1H), 4.21 (m, 1H), 3.93 (m, 3H), 3.64 (m, 3H), 3.41 (m, 4H), 3.31 (m, 2H), 3.19 (t, J = 7.5 Hz, 2H), 3.07 (m, 2H), 2.71 (s, 6H), 2.53 (m, 2H), 2.37 (m, 1H), 2.24 (s, 3H), 1.97 (m, 2H), 1.01 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 7.0 Hz, 3H); IR (neat) ν_{max} 3451, 1643, 1256, 1094, 1021 cm^{-1}; FABMS (NBA) *m*/*z* 1003 (M^+, $C_{40}H_{59}N_{16}O_9S_3$).

***N*$^{\beta}$-[1-Amino-3(*S*)-[4-amino-6-[[[1(*S*)-(((4(*S*)-(((1(*S*)-(((2-(4′-((((3-dimethylsulfonio)-1-propyl)amino)carbonyl)-2′,4-bithiazol-2-yl)-1-ethyl)amino)carbonyl)-2-methyl-1-propyl)amino)carbonyl)-3(*S*)-hydroxy-2(*R*)-pentyl)amino)carbonyl)-2(*R*)-hydroxy-2-(imidazol-4-yl)-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-*β*-aminoalanine amide (7):**[67] (83%) R_f = 0.2 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{25}_D$ −4.0 (*c* 0.05, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.20 (s, 1H), 8.10 (s, 1H), 7.68 (br s, 1H), 7.12 (br s, 1H), 5.12 (d, J = 6.5 Hz, 1H), 4.75 (d, J = 6.5 Hz, 1H), 4.14 (d, J = 7.5 Hz, 1H), 4.08 (m, 1H), 3.95 (dd, J = 5.5, 9.0 Hz, 1H), 3.91 (dd, J = 5.5, 5.5 Hz, 1H), 3.73 (m, 1H), 3.67 (m, 4H), 3.58 (dd, J = 6.0, 6.0 Hz, 2H), 3.36 (dd, J = 6.5, 6.5 Hz, 2H), 3.12 (m, 1H), 2.92 (s, 6H), 2.85 (m, 2H), 2.69 (dd, J = 4.5, 15.0 Hz, 1H), 2.51 (dq, J = 7.0, 7.0 Hz, 1H), 2.28 (s, 3H), 2.14 (tt, J = 7.0, 7.0 Hz, 2H), 2.00 (m, 1H), 1.13 (d, J = 7.0 Hz, 3H), 1.06 (d, J = 7.0 Hz, 3H), 0.89 (d, J = 4.0 Hz, 3H), 0.87 (d, J = 4.0 Hz, 3H); IR (neat) ν_{max} 3220, 2923, 1656, 1641, 1589, 1548, 1492, 1446, 1246, 1092 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1045.4215 (M^+, $C_{43}H_{65}N_{16}O_9S_3$ requires 1045.4282).

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Supporting Information Available: Experimental procedures and characterization of **10a**−**e**, **11a**−**e**, **13a**−**e**, **14a**−**e**, **15a**−**e**, **17a**−**e**, **18a**−**e**, and **20a**−**e**, experimental details of the DNA-cleavage studies, and the styrene oxidation studies are provided (14 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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